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NTP Technical Report
on Toxicity Studies of

Black Newsprint Inks

Administered Topically
to F344/N Rats and C3H Mice

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The NTP report on the toxicity studies of black newsprint ink is based primarily on 30-day and 13-week studies performed over the period of December, 1987, to September, 1988, at Battelle Memorial Laboratories, Columbus Division, Columbus, Ohio.

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ABSTRACT

Toxicity studies were conducted by applying black newsprint inks or mineral oils to clipped skin of the dorsal interscapular area of C3H mice and F344/N rats of both sexes, to determine systemic and local effects. Four lots of both letterpress and offset types of newsprint ink were studied, either as composite mixtures or as individual lots. An industrial grade mineral oil, used as an extender for newsprint ink formulation, and USP medicinal grade mineral oil also were studied. Analyses for the presence of polycyclic aromatic hydrocarbons (PAHs) were conducted on composite ink mixtures and mineral oils; letterpress and offset ink mixtures were found to have cumulative concentrations of 206 and 105 ppm, respectively; the concentration of PAHs in the printing ink mineral oil sample was 208 ppm, while none were detected in the USP grade mineral oil. In genetic toxicity studies, letterpress and offset newsprint ink composite mixtures were each mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when tested in a preincubation protocol with added hamster liver S9. With rat liver S9, results for both inks were positive in strain TA98 and negative in strain TA100. Neither type of ink was mutagenic in the absence of S9 activation.

In 30-day studies, 5 rats and mice per sex were given single, daily dermal applications of letterpress or offset newsprint inks, 5 days per week, for a total of 21 - 22 applications. Dose groups for each type of ink received either the neat (undiluted) composite ink mixture, or the 3:1, 1:1, or 1:3 dilutions (ink:USP mineral oil), with a total dose volume of 100 (mice) or 250 (rats) µl. All animals survived until the end of the studies. Toxicity attributed to ink administration was limited to decreased body weight gains in female rats treated with neat and the 3:1 dilution of letterpress ink, and to scaliness at the site of application in 1 or more mice in each letterpress ink treatment group. As a result of grooming activity and the large amount of test chemical applied, chemicals were spread over the body, and there was evidence that some oral ingestion had occurred.

In 13-week studies, various ink and mineral oil formulations were administered dermally to 10 rats and mice per sex. To prevent accumulation of inks and distribution over the body as seen in the 30-day studies, the frequency of application was reduced to twice weekly and the total dose volume was decreased to 20 microliters for mice and 50 microliters for rats. Treatment groups for rats consisted of letterpress ink mixture, offset ink mixture, printing ink mineral oil, USP mineral oil, and clipped, untreated controls. Groups of mice were administered each of the 4 individual lots of both letterpress and offset inks, the composite mixtures of each, and printing ink and USP mineral oils; clipped, untreated groups served as controls. All rats, all male mice, and all female mice except one administered offset ink-lot E survived to the end of the studies. Effects attributable to compound administration in rats were limited to decreased body weight gains in females treated with printing ink mineral oil and letterpress ink mixture, and increased liver and kidney weights in both males and females exposed to USP mineral oil; there were no local toxic effects at the site of application. In mice, there were no body weight effects, but liver weights were increased in most ink and mineral oil treatment groups of both sexes. Dermal toxicity was evidenced in mice by scaliness and irritation at the site of application of both sexes treated with USP mineral oil and letterpress ink-lot C.

Microscopically, local toxicity at the site of application was observed in mice of all treatment groups and was characterized by acanthosis and inflammation.

In summary, results of these studies indicate that topical administration of black newsprint inks and mineral oils produces local toxicity at the site of application in mice; toxic effects on the skin in this species are consistent with those of a primary cutaneous irritant. In rats, possible evidence for toxicity was limited to decreased body weight gains in females treated with letterpress ink formulations.

PEER REVIEW

Peer Review Panel

The members of the peer review panel who evaluated the draft report on the toxicity studies on black newsprint inks on July 10, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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Summary of Peer Review Comments

On July 9 and 10, 1991, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of black newsprint inks.

Dr. J.F. Mahler, NIEHS, introduced the short-term toxicity studies of black newsprint inks by reviewing the uses of and rationale for study the inks, their composition, the methodology for topical application of the inks and mineral oils, experimental design, and results.

Dr. Davis, a principal reviewer, said the study was well-designed, very complex, and had been concisely and clearly reported. He said that the report summary mentioned little if any toxicity beyond the site of topical application, yet female rats had decreased weight gains and decreased heart and lung weights, compared to controls. He asked if these effects might be attributable to decreased feed consumption. Dr. Mahler agreed that the body-weight decreases were substantial enough to be seen as evidence of toxicity and that this would be noted in the report.

Dr. Bailey, a second principal reviewer, said the experiments were well-done and the report well-written. He cited references to the work of Blackburn *et al.* on the modified Ames assay and said these references should be included in the report, since the assay can accurately differentiate between carcinogenic and non-carcinogenic mineral oils and would also provide highly reliable estimates of the relative potency of such materials in mouse skin painting bioassays. Dr. Mahler said a later reference to the modified Ames assay was cited in the report, but that the Blackburn *et al.* work would be included as well.

Dr. Bailey said it would be helpful to include the refining histories for the printing ink mineral oils used in the study, since reference was made to the fact that less-refined oils have greater carcinogenic potential. Dr. Mahler said he would seek to include the refining histories of these oils in an appendix; however, subsequent efforts to locate further information concerning the refining histories were unsuccessful.

Dr. Bailey asked why the C3H mouse was used in the study. Dr. J. Bucher, NIEHS, noted that the National Institute of Occupational Safety and Health (NIOSH) had suggested use of that strain, saying it had a data base on dermal application studies using that type of animal. However, Dr. J. Haartz, NIOSH, said this database was not large and that much of it was based on studies involving asphalt fumes and fume fractions.

Mr. Beliczky said reference should be made to the fact that carbon black, used in some black newsprint inks, contains polycyclic aromatic hydrocarbons (PAHs); he noted that the International Agency for Research on Cancer (IARC) lists some PAHs in oil as human skin carcinogens. Dr. Mahler said that references cited in the report confirm Mr. Beliczky's comments.

After discussion of editorial matters, the panel agreed to accept the report, with the indicated changes.